Association Analyses Identify Three Susceptibility Loci for Vitiligo in the Chinese Han Population

Xian-Fa Tang[1,2,3,18], Zheng Zhang[1,2,3,18], Da-Yan Hu[1,2,3,18], Ai-E Xu[4,18], Hai-Sheng Zhou[3,18], Liang-Dan Sun[1,2,3], Min Gao[1,2,3], Tian-Wen Gao[5], Xing-Hua Gao[6], Hong-Duo Chen[6], Hong-Fu Xie[7], Cai-Xia Tu[8], Fei Hao[9], Ri-Na Wu[10], Fu-Ren Zhang[11], Ling Liang[12], Xiong-Ming Pu[13], Jian-Zhong Zhang[14], Jian-Wen Han[10], Gong-Pu Pan[3], Jia-Qiang Wu[15], Kai Li[5], Ming-Wan Su[16], Wei-Dong Du[3], Wei-Jia Zhang[3,17], Jian-Jun Liu[3], Lei-Hong Xiang[15], Sen Yang[1,2,3], You-Wen Zhou[3,16] and Xue-Jun Zhang[1,2,3,15]

To identify susceptibility loci for vitiligo, we extended our previous vitiligo genome-wide association study with a two-staged replication study that included 6,857 cases and 12,025 controls from the Chinese Han population. We identified three susceptibility loci, 12q13.2 (rs10876864, $P_{combined} = 8.07 \times 10^{-12}$, odds ratio (OR) = 1.18), 11q23.3 (rs638893, $P_{combined} = 2.47 \times 10^{-9}$, OR = 1.22), and 10q22.1 (rs1417210, $P_{combined} = 1.83 \times 10^{-8}$, OR = 0.88), and confirmed three previously reported loci for vitiligo, 3q28 (rs9851967, $P_{combined} = 8.57 \times 10^{-8}$, OR = 0.88), 10p15.1 (rs3134883, $P_{combined} = 1.01 \times 10^{-5}$, OR = 1.11), and 22q12.3 (rs2051582, $P_{combined} = 2.12 \times 10^{-5}$, OR = 1.14), in the Chinese Han population. The most significant single-nucleotide polymorphism in the 12q13.2 locus is located immediately upstream of the promoter region of *PMEL*, which encodes a major melanocyte antigen and has expression loss in the vitiligo lesional skin. In addition, both 12q13.2 and 11q23.3 loci identified in this study are also associated with other autoimmune diseases such as type 1 diabetes and systemic lupus erythematosus. These findings provide indirect support that vitiligo pathogenesis involves a complex interplay between immune regulatory factors and melanocyte-specific factors. They also highlight similarities and differences in the genetic basis of vitiligo in Chinese and Caucasian populations.

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INTRODUCTION

Vitiligo is an autoimmune disease presenting with progressive loss of skin pigmentation affecting about 1% of individuals in most populations worldwide (Le Poole and Luiten, 2008). Generalized vitiligo is an acquired, noncontagious disorder characterized by progressive, patchy loss of pigmentation of skin, and often overlying hair and mucous membranes, resulting from loss of melanocytes in the affected areas (Spritz, 2007). Clinical and epidemiological investigations indicated that vitiligo might follow a pattern of polygenetic or multifactorial inheritance (Alkhateeb *et al.*, 2003; Zhang *et al.*, 2004). Many repigmentation therapies, such as steroids and UV irradiation, are immunosuppressive and the beneficial effect of such treatments supports the involvement of autoimmunity in vitiligo. Association between vitiligo and other autoimmune diseases also

[1]*Department of Dermatology and Venereology, Anhui Medical University, Hefei, China;* [2]*Institute of Dermatology and Department of Dermatology at No.1 Hospital, Anhui Medical University, Hefei, China;* [3]*State Key Laboratory Incubation Base of Dermatology, Ministry of National Science and Technology, Anhui, China;* [4]*Department of Dermatology, The Third People's Hospital of Hangzhou, Hangzhou, China;* [5]*Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, China;* [6]*Department of Dermatology, No.1 Hospital of China Medical University, Shenyang, China;* [7]*Department of Dermatology, Xiangya Hospital, Central South University, Changsha, China;* [8]*Department of Dermatology, The 2nd Affiliated Hospital of Dalian Medical University, Dalian, China;* [9]*Department of Dermatology, Southwest Hospital, Third Military Medical University, Chongqing, China;* [10]*Department of Dermatology, The Affiliated Hospital of Inner Mongolia Medical College, Huhehot, China;* [11]*Department of Dermatology, Shandong Provincial Institute of Dermatology and Venereology, Jinan, China;* [12]*Department of Dermatology, The First Affiliated Hospital of Guangxi Medical University, Nanning, China;* [13]*Department of Dermatology, People's Hospital of Xinjiang Uygur Autonomous Region, Urumchi, China;* [14]*Department of Dermatology, Peking University People's Hospital, Beijing, China;* [15]*Department of Dermatology, Huashan Hospital of Fudan University, Shanghai, China;* [16]*Department of Dermatology and Skin Science, University of British Columbia, Vancouver, British Columbia, Canada and* [17]*Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA*

[18]*These authors contributed equally to this work.*

Correspondence: Lei-Hong Xiang, Department of Dermatology, Huashan Hospital of Fudan University, 12 Urumqizhong Road, Shanghai, China. E-mail: flora_xiang@vip.163.com or You-Wen Zhou, Department of Dermatology and Skin Science, University of British Columbia, 835 West 10th Ave, Vancouver, British Columbia V5Z 4E8, Canada. E-mail: Youwen.Zhou@vch.ca or Xue-Jun Zhang, Institute of Dermatology, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China. E-mail: ayzxj@vip.sina.com

Abbreviations: GWAS, genome-wide association study; LD, linkage disequilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism

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supports the role of autoimmunity of the disease (Jin *et al.*, 2010a).

To date, a number of genetic susceptibility factors have been identified through linkage and association studies (Chen *et al.*, 2005; Ren *et al.*, 2009; Spritz, 2011); however, only a few genes/loci, such as *NALP1* (Jin *et al.*, 2007) and some *HLA* alleles (Zamani *et al.*, 2001; Arcos-Burgos *et al.*, 2002), have been consistently replicated in multiple studies. Notably, several recent genome-wide association studies (GWASs) of vitiligo in the Caucasian population have identified a number of susceptibility genes/loci (Jin *et al.*, 2010a, 2010b, 2012), such as 1p13.2 (*PTPN22*), 1p36.23 (*RERE*), 2q24.2 (*IFIH1*), 3p13 (*FOXP1*), 3q13.33 (*CD80*), 3q28 (*LPP*), 6p21.3 (*HLA-A*), 6q27 (*CCR6*), 10p15.1 (*IL2RA*), 11p13 (*CD44*), 11q14.3 (*TYR*), 12q24.12 (*SH2B3*), 12q13.2 (*IKZF4*), 14q12 (*GZMB*), 16q24.3 (*MC1R*), 21q22.3 (*UBASH3A*), 22q13.1 (*C1QTNF6*), and 22q13.2 (*TOB2*). In the Chinese population, the identified susceptibility genes/loci included 6p21.33 (*HLA-B* and *HLA-C*), 6q27 (*RNASET2*, *FGFR1OP*, and *CCR6*), and 10q22.3 (*ZMIZ1*) (Quan *et al.*, 2010). Most of these loci encode immune-related proteins and several have been associated with other autoimmune diseases, highlighting common immune pathogenetic pathways underlying these different disorders.

In this study, to identify additional susceptibility loci for vitiligo, we expanded our previous vitiligo GWAS (Quan *et al.*, 2010), increasing the power of both the genome-wide discovery stage and subsequent replication stage by adding 272 healthy controls to the previous genome-wide screening stage for a total of 1,117 cases and 1,701 controls, with replication in two independent cohorts (replication 1: 2,827 cases and 3,876 controls; replication 2: 2,913 cases and 6,448 controls; Table 1). In addition, we conducted gene expression studies on the genes implicated in the loci identified in the current study and the genes implicated in previous vitiligo GWASs to provide additional information on the genes implicated in vitiligo genetic pathogenesis.

RESULTS AND DISCUSSION

Our GWAS data set consisted of 620,901 single-nucleotide polymorphisms (SNPs) and copy number variant probes genotyped in 1,117 cases and 1,701 controls in the current study. After quality control, the genotype data of 493,909 autosomal SNPs in 1,117 cases and 1,701 controls were used for association testing (Figure 1a). The quantile–quantile (Q–Q) plots of the logarithms of our genome-wide *P*-values, after removal of 4,031 SNPs from the major histocompatibility complex region (6p21: 25–34 Mb), largely fit the null distribution, except at the tail of the distribution (Figure 1b). Principal component analysis indicated that the cases and controls were genetically matched (Supplementary Figure S1 online), and a moderate genomic inflation factor of $\lambda gc = 1.07$ indicated minimal overall population stratification.

We employed three strategies for selecting SNPs (see Materials and Methods). In total, 50 SNPs at 44 loci were selected for the replication testing (replication 1: 2,827 cases and 3,876 controls) (Supplementary Table S1 and S3–5 online). After applying standard quality control procedures, the 13 most significant of the 50 SNPs ($P_{\text{replication 1}} < 0.05$) were further genotyped in a second independent replication cohort (replication 2: 2,913 cases and 6,448 controls) (Supplementary Table S5 online). These analyses identified three loci, 12q13.2 (rs10876864, $P_{\text{combined}} = 8.07 \times 10^{-12}$, odds ratio (OR) = 1.18), 11q23.3 (rs638893, $P_{\text{combined}} = 2.47 \times 10^{-9}$, OR = 1.22), and 10q22.1 (rs1417210, $P_{\text{combined}} = 1.83 \times 10^{-8}$, OR = 0.88), reaching genome-wide significance in the combined analysis of the GWAS and two replication samples (Table 2). In addition, we also confirmed three previously reported vitiligo susceptibility loci, 3q28 (rs9851967 and rs13076312), 10p15.1 (rs3134883), and 22q12.3 (rs2051582), with suggestive evidence of association ($8.57 \times 10^{-8} \leqslant P_{\text{combined}} \leqslant 2.12 \times 10^{-5}$, Table 2). Results for the other tested SNPs are summarized in Supplementary Table S5 online.

Rs10876864, the most strongly associated SNP at the 12q13.2 locus, is located in a linkage disequilibrium (LD) block that contains multiple genes (Figure 2a). It has strong LD with the reported SNP rs1701704 ($r^2 = 0.94$ in the CHB + JPT panel) that is located upstream of *IKZF4* (encoding a zinc-finger transcription factor of T-cell activation, which is a critical coregulator of FoxP3-directed gene silencing during $CD4^+$ regulatory T-cell differentiation (Pan *et al.*, 2009)) in previous type 1 diabetes (Hakonarson *et al.*, 2008), alopecia areata (Petukhova *et al.*, 2010), and vitiligo (Jin *et al.*, 2012) studies. Interestingly, the associated SNP rs10876864 is located 40 kb centromeric to *PMEL*, which encodes a melanocyte protein necessary for eumelanin deposition 1 in mammals (McGlinchey *et al.*, 2009). It is shown that the antigen-specific $CD8^+$ T cells exhibit reactivity to modified PMEL peptide epitopes in HLA-A2-positive vitiligo patients,

Table 1. Sample summary information for the GWAS and replication studies

		Cases			Controls		
Analysis	Population	Sample size	Mean age (SD)	Male/female	Sample size	Mean age (SD)	Male/female
GWAS	Chinese Han	1,117	28.73 (±15.00)	656/461	1,701	35.9 (±14.81)	928/773
Replication 1	Chinese Han	2,827	26.91 (±14.35)	1,438/1,389	3,876	28.43 (±10.44)	1,890/1,986
Replication 2	Chinese Han	2,913	28.25 (±14.82)	1,564/1,349	6,448	29.39 (±15.86)	3,283/3,165
Total		6,857	27.36 (±14.54)	3,658/3,199	12,025	30.33 (±14.51)	6,101/5,924

Abbreviation: GWAS, genome-wide association study.

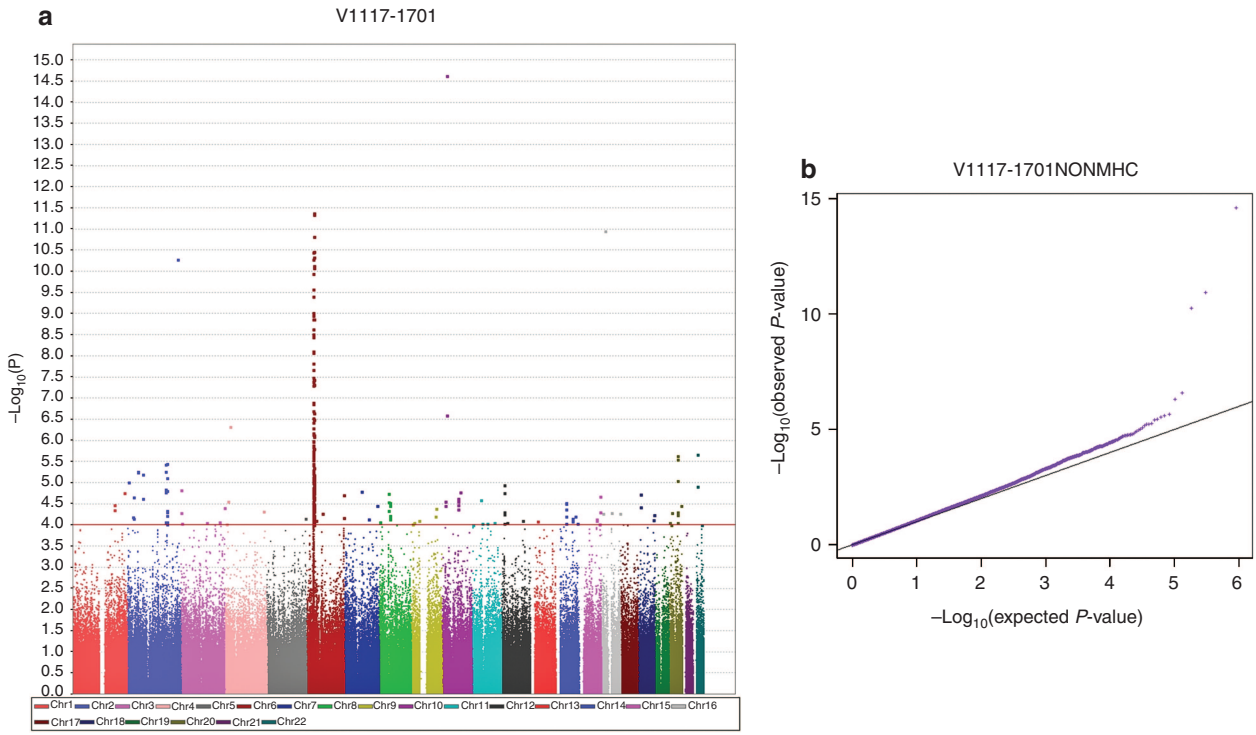

Figure 1. Genome-wide association results from the initial genome-wide association study (GWAS) analysis. (**a**) The genome-wide *P*-values of the Cochran–Armitage trend test from 493,909 polymorphic single-nucleotide polymorphisms (SNPs) in 1,117 vitiligo cases and 1,701 controls of the Chinese Han ancestry are presented. The chromosomal distribution of all the *P*-values ($-\log_{10}$ *P*-values) is shown. (**b**) Quantile–quantile (Q–Q) plots of the observed *P*-values for association. The gray line is for null expectation. The plot in purple is for *P*-values for SNPs excluding the 4,031 SNPs within the major histocompatibility complex (MHC) region (6p21: 25–34 Mb).

Table 2. Association evidence for seven SNPs at six loci in the GWAS, the replication studies, and the combined analysis in the Chinese Han population

			GWAS 1,117 cases, 1,701 controls				Replication study 1 2,827 cases, 3,876 controls				Replication study 2 2,913 cases, 6,448 controls				Combined Chinese Han 6,857 cases, 12,025 controls		
			MAF				MAF				MAF						
SNP	Chr	Allele	Case	Control	OR	*P*[1]	Case	Control	OR	*P*[1]	Case	Control	OR	*P*[1]	OR (95% CI)	*P*[1]	Gene
Identified loci																	
rs10876864	12q13.2	G	0.29	0.25	1.21	1.62E−03	0.26	0.23	1.17	8.55E−05	0.27	0.24	1.18	2.52E−06	1.18 (1.13–1.24)	8.07E−12	Multiple genes
rs638893	11q23.3	C	0.13	0.10	1.31	1.40E−03	0.13	0.11	1.23	1.38E−04	0.13	0.11	1.18	7.12E−04	1.22 (1.14–1.30)	2.47E−09	*CXCR5/DDX6*
rs1417210	10q22.1	C	0.27	0.32	0.78	4.04E−05	0.29	0.32	0.87	3.01E−04	0.29	0.31	0.91	8.56E−03	0.88 (0.84–0.92)	1.83E−08	*SLC29A3/CDH23*
Confirmed known loci																	
rs9851967	3q28	T	0.26	0.31	0.81	4.93E−04	0.26	0.29	0.87	5.98E−04	0.27	0.28	0.91	8.08E−03	0.88 (0.84–0.92)	8.57E−08	*LPP*
rs13076312	3q28	C	0.32	0.37	0.81	2.28E−04	0.32	0.34	0.92	3.58E−02	0.32	0.34	0.92	1.52E−02	0.90 (0.86–0.94)	9.37E−06	*LPP*
rs3134883	10p15.1	T	0.32	0.29	1.16	1.58E−02	0.30	0.28	1.09	2.76E−02	0.31	0.28	1.11	2.34E−03	1.11 (1.06–1.16)	1.01E−05	*IL2RA*
rs2051582	22q12.3	A	0.17	0.14	1.24	4.52E−03	0.16	0.14	1.14	8.85E−03	0.16	0.15	1.10	2.74E−02	1.14 (1.07–1.21)	2.12E−05	*IL2RB*

Abbreviations: Chr, chromosome; CI, confidence interval; GWAS, genome-wide association study; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.
[1]Two-tailed *P*-values from the Cochran–Armitage trend test.

which also supports the notion that there is a cell-mediated immunopathologic mechanism in vitiligo (Mandelcorn-Monson *et al.*, 2003). By skin biopsy transcriptome analysis, *PMEL* was shown to have the strongest differential expression between vitiligo lesional skin and vitiligo perilesional normal skin, with a 5.94-fold decrease in *PMEL* expression in lesional

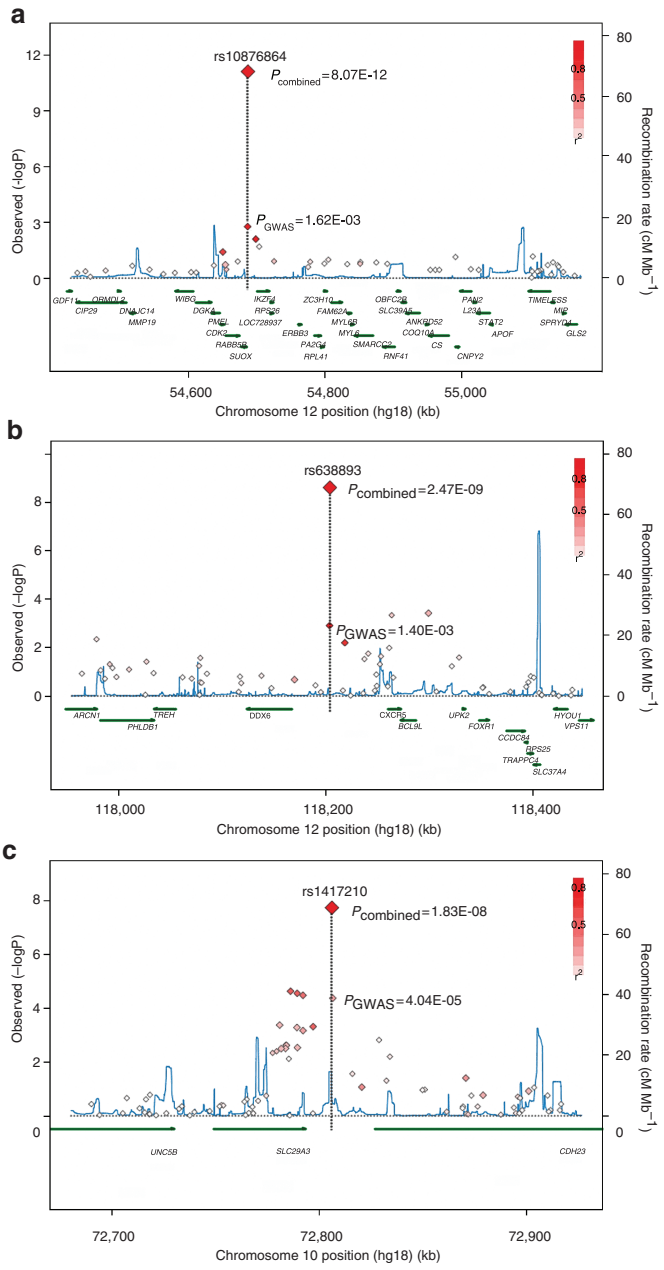

Figure 2. Association scatter plots for three susceptibility loci. According to the genome-wide single-marker association analysis using the Cochran–Armitage trend test, the *P*-values of single-nucleotide polymorphisms (SNPs) (shown as $-\log_{10}$ values in *y*-axis) were plotted against their map positions (*x*-axis). The color of each SNP spot reflects its r^2 with the top SNP (large red diamond) within each association locus, changing from red to white. Estimated recombination rates (based on the combined CHB and JPT samples from the HapMap project) were plotted in light blue. Gene annotations were adapted from the University of California Santa Cruz Genome Browser (http://genome.ucsc.edu/). (**a**) 12q13.2, (**b**) 11q23.3, and (**c**) 10q22.1.

skin compared with perilesional normal skin ($P_{Bonferroni} = 2.91 \times 10^{-6}$, Table 3). It has been postulated that *PMEL* might have an important role in the structural organization of premelanosomes, suggesting its potential role in the pathogenesis of vitiligo.

It is intriguing that two other genes encoding major melanocyte antigens, *TYR* and *OCA2*, have been strongly implicated in vitiligo GWAS in the Caucasian population (Jin *et al.*, 2010a, 2012). Both genes also showed strong expression loss in the lesional skin of vitiligo (Table 3). Interestingly, neither of these genes emerges in the studies on the Chinese population. Thus, there appears to be a difference between the Caucasians and Chinese with respect to the melanocyte-specific antigens involved in the pathogenesis of vitiligo, although further studies are needed to elucidate this apparent difference.

SNP rs638893 at 11q23.3 was located in an intergenic region between *DDX6* and *CXCR5* (Figure 2b). It has weak LD with the reported SNPs rs4639966 ($r^2 = 0.10$ in the CHB + JPT panel) and rs10892279 ($r^2 = 0.38$ in the CHB + JPT panel) near the *DDX6* gene in previous systemic lupus erythematosus, celiac disease, and rheumatoid arthritis studies (Han *et al.*, 2009; Zhernakova *et al.*, 2011). *DDX6* encodes a member of the DEAD box protein family, which is an RNA helicase found in P-bodies and stress granules and functions in translation suppression and mRNA degradation (Weston and Sommerville, 2006). For this locus, another candidate gene *CXCR5* encodes the receptor for CXC-chemokine ligand 13, a chemokine that is secreted by *CXCR5* follicular stromal cells. A defining molecule for T cells with follicular-homing capability which are specialized for providing help to germinal center B cells during antibody responses to T-cell–independent antigens. *CXCR5* has been shown to have an important role in the pathogenesis of autoimmunity (Vinuesa *et al.*, 2005; Morita *et al.*, 2011).

The associated SNP rs1417210 at 10q22.1 is located in an LD block that contains *SLC29A3* and *CDH23* (Figure 2c). According to the Chinese and Japanese data from the 1000 Genome Project, one nonsynonymous SNP rs2252996 in *SLC29A3* (G→A, V239I) has moderate LD ($r^2 = 0.60$) with rs1417210, and further imputation analysis revealed the stronger association at rs2252996 ($P_{imputed} = 2.33 \times 10^{-5}$ using GWAS sample) than at rs1417210 ($P_{GWAS} = 4.04 \times 10^{-5}$). *SLC29A3* encodes the equilibrative nucleoside transporter hENT3, and mutations in *SLC29A3* have been found to be associated with the H syndrome, which can include cutaneous hyperpigmentation (Molho-Pessach *et al.*, 2008). It is likely that the causal variant alters *SLC29A3* gene functions through other mechanisms. Another candidate gene of interest, *CDH23*, belongs to a member of the cadherin superfamily, which encodes calcium-dependent cell–cell adhesion glycoproteins.

Of the four previously reported vitiligo susceptibility loci analyzed in our study, two loci, 3q28 (*LPP*) and 10p15.1 (*IL2RA*), were confirmed in the Chinese Han population (Table 2, Supplementary Figure S2a and S2b online). However, we did not observe significant associations for the SNPs rs830605 at 3p13 and rs5756546 at 22q13.1 in the Chinese Han population ($P_{combined} > 0.04$, Supplementary Table S6 online). Interestingly, we observed an associated signal on 22q12.3 (rs2051582, Table 2). SNP rs2051582 resides between *IL2RB* (22q12.3) and *C1QTNF6* (22q13.1) (Supplementary Figure S2c online). *IL2RB* has been found to

Table 3. Lesional expression changes of genes located in the six loci in this study and the genes implicated in previous vitiligo GWASs

Cyto-band	Population	Gene symbol	Vit LS	Vit NS	Ratio (LS/NS)	*P**	*P***	Reference
3q28	CHI	*LPP*	2,254	2,314	0.97	0.80	>0.05	Current study
10p15.1	CHI	*IL2RA*	131	56	2.32	0.22	>0.05	Current study
10p15.1	CHI	*RBM17*	9,622	9,439	1.02	0.53	>0.05	Current study
10p15.1	CHI	*PFKFB3*	4,132	4,992	0.83	0.01	>0.05	Current study
10q21.2	CHI	*SLC16A9*	154	157	0.98	0.92	>0.05	Current study
10q21.2	CHI	*CCDC6*	35,834	33,542	1.07	0.05	>0.05	Current study
10q21.2	CHI	*ANK3*	16,578	16,567	1.00	0.99	>0.05	Current study
10q21.2	CHI	*CDC2*	1,260	960	1.31	0.00	>0.05	Current study
10q21.2	CHI	*TMEM26*	24	25	0.96	0.63	>0.05	Current study
10q22.1	CHI	*UNC5B*	6,983	10,419	0.67	0.01	>0.05	Current study
10q22.1	CHI	*SLC29A3*	312	309	1.01	0.90	>0.05	Current study
10q22.1	CHI	*CDH23*	117	139	0.84	0.35	>0.05	Current study
11q23.3	CHI	*PHLDB1*	622	812	0.77	0.00	>0.05	Current study
11q23.3	CHI	*TREH*	22	23	0.94	0.18	>0.05	Current study
11q23.3	CHI	*AK021715*	3,082	3,592	0.86	0.01	>0.05	Current study
11q23.3	CHI	*DDX6*	853	794	1.07	0.26	>0.05	Current study
12q13.2	CHI	*DGKA*	1,689	1,893	0.89	0.08	>0.05	Current study
12q13.2	**CHI**	**PMEL**	**2,556**	**15,178**	**0.17**	**7.09E−11**	**2.91E−06**	Current study
12q13.2	CHI	*CDK2*	163	199	0.82	0.00	>0.05	Current study
12q13.2	CHI	*RAB5B*	2,429	2,644	0.92	0.07	>0.05	Current study
12q13.2	CHI	*SUOX*	354	459	0.77	0.01	>0.05	Current study
12q13.2	CHI	*ZNFN1A4*	714	518	1.38	0.00	>0.05	Current study
12q13.2	CHI	*RPS26*	136,513	131,924	1.03	0.12	>0.05	Current study
12q13.2	CHI	*ERBB3*	4,520	5,062	0.89	0.07	>0.05	Current study
12q13.2	CHI	*PA2G4*	7,964	7,329	1.09	0.04	>0.05	Current study
22q13.1	CHI	*IL2RB*	897	891	1.01	0.95	>0.05	Current study
1p13.2	CAU	*PTPN22*	67	65	1.01	0.91	>0.05	Jin *et al.* (2010a)
1p36.23	CAU	*RERE*	640	645	0.99	0.76	>0.05	Jin *et al.* (2010a)
2q24.2	CAU	*IFIH1*	2,133	1,989	1.07	0.22	>0.05	Jin *et al.* (2012)
3p13	CAU	*FOXP1*	13,893	14,408	0.97	0.32	>0.05	Jin *et al.* (2010b)
3q13.33	CAU	*CD80*	29	22	1.27	0.00	>0.05	Jin *et al.* (2012)
6p21.32	CAU	*C6orf10*	22	18	1.12	0.20	>0.05	Jin *et al.* (2011)
6p21.32	CAU	*BTNL2*	207	191	1.11	0.06	>0.05	Jin *et al.* (2011)
6p21.32	CAU	*HLA-DRA*	24,168	26,279	0.90	0.22	>0.05	Jin *et al.* (2010a)
6p21.32	CAU	*HLA-DQA1*	3,251	3,553	0.80	0.04	>0.05	Jin *et al.* (2010a)
6p21.33	CHI	*HLA-C*	180,807	200,667	0.91	0.19	>0.05	Quan *et al.* (2010)
6p21.33	CHI	*HLA-B*	87,298	113,152	0.76	0.00	>0.05	Quan *et al.* (2010)
6p22.1	CAU	*HLA-A*	29,884	37,321	0.80	0.00	>0.05	Jin *et al.* (2010a)
6p22.1	CAU	*HCG9*	23	22	1.02	0.86	>0.05	Jin *et al.* (2010a)
6q15	CAU	*BACH2*	477	490	0.97	0.61	>0.05	Jin *et al.* (2012)
6q27	CHI and CAU	*CCR6*	341	275	1.05	0.76	>0.05	Jin *et al.*, 2010b; Quan *et al.*, 2010
6q27	CHI	*RNASET2*	6,585	6,503	1.00	1.00	>0.05	Quan *et al.* (2010)

Table 3 continued on following page

Table 3. Continued

Cyto-band	Population	Gene symbol	Vit LS	Vit NS	Ratio (LS/NS)	*P**	*P***	Reference
6q27	CHI	*FGFR1OP*	822	621	1.34	0.00	>0.05	Quan *et al.* (2010)
6q27	CAU	*SMOC2*	13,074	17,624	0.84	0.11	>0.05	Birlea *et al.* (2010)
8q24.22	CAU	*SLA*	5,403	4,969	1.06	0.49	>0.05	Jin *et al.* (2012)
10q22	CHI	*ZMIZ1*	9,422	8,289	1.12	0.00	>0.05	Quan *et al.* (2010)
10q25.3	CAU	*CASP7*	4,678	4,418	1.04	0.50	>0.05	Jin *et al.* (2012)
11p13	CAU	*CD44*	44,395	43,993	1.01	0.81	>0.05	Jin *et al.* (2012)
11q14.3	**CAU**	**TYR**	**232**	**6,691**	**0.02**	**2.96E−09**	**1.21E−04**	Jin *et al.* (2010a)
12q13.2	CAU	*IKZF4*	699	513	1.34	0.00	>0.05	Jin *et al.* (2012)
12q24.12	CAU	*SH2B3*	320	381	0.85	0.02	>0.05	Jin *et al.* (2012)
14q12	CAU	*GZMB*	593	485	1.18	0.40	>0.05	Jin *et al.* (2010a)
15q12-13.1	**CAU**	**OCA2**	**440**	**1,246**	**0.35**	**1.84E−07**	**7.54E−03**	Jin *et al.* (2012)
15q12-13.1	CAU	*HERC2*	2,261	2,546	0.89	0.02	>0.05	Jin *et al.* (2012)
16q24.3	CAU	*MC1R*	1,551	2,565	0.59	0.00	>0.05	Jin *et al.* (2012)
22q13.2	CAU	*TOB2*	3,886	3,959	0.98	0.58	>0.05	Jin *et al.* (2012)
22q12.3	CAU	*C1QTNF6*	651	818	0.84	0.02	>0.05	Jin *et al.* (2010a)
21q22.3	CAU	*UBASH3A*	80	78	1.03	0.77	>0.05	Jin *et al.* (2010a)

Abbreviations: CAU, Caucasian; CHI, Chinese; GWAS, genome-wide association study; Vit LS, vitiligo lesional skin; Vit NS, vitiligo perilesional normal skin.
**P*-value using paired Student *t*-test was used to calculate the statistical significance before correction for multiple testing.
***P*-value after multiple testing with Bonferroni method using GeneSpring GX7.3 software.
Characters written in bold indicate that genes had significantly different expression.

be associated with rheumatoid arthritis in the Caucasian population (Eleftherohorinou *et al.*, 2011). *C1QTNF6* has been shown to be associated with other autoimmune diseases including vitiligo (Jin *et al.*, 2010a). The three confirmed loci (3q28, 10p15.1, and 22q12.3) in this study have clearly supported the existence of common disease susceptibility for vitiligo between the Chinese and Caucasian populations.

Using the restricted maximum likelihood method (Yang *et al.*, 2010), we estimated that the six loci explained nearly 1% of the overall variance in disease risk. GWASs, which have generally focused on common SNPs, have been completed for most complex diseases. However, the causal SNPs may lie within a much larger region than the LD block surrounding the associated common SNPs (Cirulli and Goldstein, 2010). Thus, it is speculated that the gene(s) located in the adjacent LD block may also be potential candidate one(s) for diseases. Therefore, further efforts (fine mapping and functional study) are needed to determine the causal gene(s) within each locus for vitiligo.

In summary, we conducted an extended GWAS on vitiligo in the Chinese Han population and identified three disease susceptibility loci and also confirmed three previously reported loci. A melanocyte-specific antigen-encoding gene, *PMEL*, might be implicated in the pathogenesis of vitiligo in the Chinese population. Not only does this observation indirectly support the pathogenic significance of immunological interaction between the immune-regulating genes and the melanocyte-specific genes in vitiligo development, but it also provides further evidence that population-specific differences exist between the Caucasian and Chinese populations in the genetic predisposition to vitiligo.

MATERIALS AND METHODS

Subjects

All samples were recruited from the Chinese Han population through collaboration with multiple hospitals in China (Quan *et al.*, 2010). The clinical diagnosis of each patient was confirmed by at least two dermatologists according to the diagnostic criteria of Vitiligo European Task Force (Taieb and Picardo, 2007). We collected clinical information from the subjects through a full clinical checkup, and additional demographic information from both the cases and controls was obtained through a structured questionnaire as used previously (Quan *et al.*, 2010). A written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of the Anhui Medical University and was conducted according to the Declaration of Helsinki principles.

SNP selection for replication

We adopted three approaches to select SNPs for replication. First, after excluding previously reported vitiligo risk alleles and all SNPs within the extended major histocompatibility complex region (6p21: 25–34 Mb), we selected SNP(s) at each locus that showed suggestive association ($P_{initial} < 1 \times 10^{-4}$) after stringent quality control procedures. We selected the 19 most strongly associated SNPs at 15 loci for replication testing (Supplementary Table S1 online). Second, we searched the published GWAS on other autoimmune diseases from the GWAS catalog (including ankylosing spondylitis, amyotrophic lateral sclerosis, type 1 diabetes , inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, psoriasis, systemic

lupus erythematosus, and ulcerative colitis; Supplementary Table S2 online) and summarized the previously reported susceptibility loci for these autoimmune diseases. Using HapMap data, we then searched the LD blocks tagged by these reported top SNPs for related autoimmune diseases and then identified the top SNPs in these blocks from our own vitiligo GWAS for further replication (For European GWAS results, we defined the LD block based on the CEU panel in HapMap 2 and 3; for Asian GWAS result, analyses were based on JPT and CHB panels of HapMap 2 and on CHB, CHD, JPT panels of HapMap 3). All the SNPs in the LD block have $r^2 \geqslant 0.2$ with the top SNPs, and we constrained LD blocks to 500 kb. A total of 26 SNPs in 25 autoimmune disease loci that showed nominal evidence of association ($P_{initial} < 0.05$) in our vitiligo GWAS were selected for replication (Supplementary Table S3 online). Finally, we selected five SNPs ($P_{initial} < 0.05$) from four vitiligo susceptibility loci previously reported in the Caucasian population (Jin *et al.*, 2010a) to determine whether these loci also constitute vitiligo susceptibility loci in the Chinese population (Supplementary Table S4 online).

Genotyping and quality control

DNA preparation, genome-wide genotyping using the Illumina 610-Quad BeadChip (Illumina, San Diego, CA) and replication genotyping using the Sequenom MassArray iPLEX system (Sequenom, San Diego, CA), and quality control filtering of the data have been described previously (Quan *et al.*, 2010).

Statistical analysis

Both the genome-wide and replication analyses were carried out using PLINK version 1.07. (Purcell *et al.*, 2007). The quantile–quantile plot and genomic inflation factor were calculated using the statistical analysis program R (http://www.r-project.org/), which evaluated the overall significance of the genome-wide association results. All statistical results were reported without genomic control correction, as minimal evidence of population stratification was found. The data set was additionally assessed for population outlier and stratification using a principal component analysis–based approach (Pe'er *et al.*, 2008; Quan *et al.*, 2010). GWAS and replication analyses were carried out using the Cochran–Armitage trend test. Calculations of association *P*-values in the GWAS, replication 1, replication 2, and combined analyses were made using two-tailed tests. Recombination plots of each discovered susceptibility locus were generated using the information from the HapMap project (CHB and JPT samples). Imputation of SNPs in our GWAS data was performed using data from the 1000 Genomes Project by Impute v2.0 (Department of Statistics, University of Oxford, UK), which was then tested for association.

Skin biopsy transcriptome analysis

RNA samples from 17 pairs of full-thickness vitiligo skin (lesional and normal perilesional skin) were used in DNA microarray analysis following a previously described protocol (Wang *et al.*, 2011; Zhang *et al.*, 2012). In brief, total cellular RNA (500 ng) was reverse-transcribed into complementary DNA and linearly amplified by *in vitro* transcription in the presence of fluorescent-labeled CTP using the Low RNA Input Linear Amplification Kit, PLUS, Two-Color, from Agilent (Agilent Technologies, Santa Clara, CA) following the manufacturer's instructions. Each microarray was hybridized with 825 ng of each amplified complementary DNA labeled with Cy5 or Cy3 at a specific activity between 8 and 15 pmol μg^{-1}. Hybridizations were performed on Whole Human Genome Oligo microarrays (G4112F, Agilent Technologies), comprising 41,059 60-nucleotide oligonucleotide probes, mostly represented as single spots. Image scanning was performed using the Agilent DNA Microarray Scanner and quantified using Agilent's Feature Extraction software. The results were imported and analyzed with GeneSpring GX 7.3 software (Agilent Technologies) for statistical computation and visualization. Data normalization was performed within and across the arrays using per-gene, per-chip normalization according to the Agilent recommendation. To present the differentially expressed genes between vitiligo lesional skin and perilesional normal skin from patients with vitiligo, paired Student *t*-tests were used to calculate the statistical significance of any expression difference found. The genes were ranked according to their false discovery rate–adjusted *P*-values and a cutoff of $P < 0.05$ (Bonferroni correction was used to control for multiple testing).

Nonsynonymous SNP analysis

Data from the 1000 Genomes Project March 2010 release (see URLs, http://www.1000genomes.org/) were used to find nonsynonymous, splice, or stop-encoding SNPs in high LD ($r^2 \geqslant 0.5$).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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